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A further new species in the lichen genus *Arctomia*: *A. borbonica* from Reunion (Mascarene archipelago)

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Abstract

Arctomia borbonica **sp. nov.** is described as new for science from montane natural and secondary habitats in Reunion in the Mascarene archipelago (Indian Ocean). It has a sterile, foliose, usually wrinkled, thallus whose margins produce goniocysts that disintegrate into a soredioid margin; it looks like a *Leptogium* species. Its phylogenetic position in the Arctomiaceae (Ostropomycetidae, Ascomycota) has been determined with 3 genes (nuLSU, mtSSU, *RPB1*) inferences.

Key words

Ascomycota, Ostropomycetidae, Arctomiaceae, *Arctomia*, phylogenetic inferences, nuLSU, mtSSU, *RPB1*, Reunion, Mascarene archipelago

Introduction

Within the Lecanoromycetes, the subclass Ostropomycetidae Reeb, Lutzoni and Cl. Roux exhibits an impressive diversity of ascomata, thallus forms and ecological requirements. The phylogenetic relationships between genera and families are poorly resolved (Baloch et al. 2010), although impressive progress has been recently achieved for the Graphidaceae (incl. Thelotremataceae), the second largest family of lichenized fungi (Rivas Plata et al. 2012). Many taxa within the subclass still require detailed phylogenetic studies. Indeed, modern statistical methods within a phylogenetical context using several loci sequences yielded interesting and quite unexpected results, such as the polyphyly of two well-known genera. *Graphis* is now resolved into two strongly

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supported clades, nested within a large clade comprizing other well-known genera such as *Diorygma, Glyphis* and *Phaeographis* (tribe Graphideae; Rivas Plata et al. 2011). Further, *Pertusaria* is resolved into four strongly supported groups: *Pertusaria* s. str. (incl. the type species *P. pertusa*), *Pertusaria* s. l. 1 including *P. amara*, *P.* s. l. 2 including *P. lactea* and *P. velata*, and a fourth group, comprizing the species with gyalectoid ascomata and recently recognized as the new genus *Gyalectaria* (Schmitt et al. 2010).

Within such a large and very much unresolved variation, the case of the Arctomiaceae is rather simple. The family is strongly supported and includes three genera: *Gregorella* and *Wawea,* each with one species, and *Arctomia* with five species (Henssen 1969; Henssen and Kantvilas 1985; Jørgensen 2003, 2007; Lumbsch et al. 2005; Øvstedal and Gremmen 2001, 2006). They are lichenized with the cyanobacteria genus *Nostoc*, have a corticate thallus, gymnocarpous ascomata, asci with a non-amyloid thallus, and 1-10-septate, hyaline ascospores.

We here report the discovery of a further new species, which we assign to the genus *Arctomia*, found epiphytic in montane habitats in the island of Reunion (Mascarene archipelago, Indian Ocean). The material was first assigned to *Leptogium*, a genus belonging to the Collemataceae in the Lecanoromycetidae (Lumbsch and Huhndorf 2010). It is an unusual species as it has a foliose, sometimes very much crumpled, thallus, producing corticate and easily detached « goniocysts », best developed at the lobes margins, disrupting when mature and then forming a soredioid margin. Three loci were amplified (nuLSU, mtSSU, *RPB1*) and inferences from the sequences produced from two collections left no doubt that the material belongs to the Arctomiaceae, and statistical support to include it in the genus *Arctomia* was found. A new species is thus described in this genus.

Methods

Well-preserved lichen specimens lacking any visible symptoms of fungal infection were used for DNA isolation. Extraction of DNA and PCR amplification were performed following the protocol of Cubero et al. (1999). The primers used were: for nuLSU, LR0R, LR3R, LR3, LR5R and LR6 (following the suggestions available on www.lutzonilab.net/primers), for mtSSU, mtSSU1 and mtSSU3R (Zoller et al. 1999), for *RPB1*, AFasc and 6R1asc (following the suggestions available on www.lutzonilab.net/primers). Amplicons were sequenced by Macrogen®. Sequence fragments were assembled with Sequencher version 4.9 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were subjected to megaBLAST searches (Wheeler et al. 2006) to detect potential contaminations.

We assembled matrices with most representatives of species included by Lumbsch et al. (2005) in their description of the new genus *Gregorella*, resolved within the strongly supported Arctomiaceae; we further added several other species belonging to the Ostropomycetidae included in the study of the gyalectoid representatives of *Pertusaria* s.l. by Schmitt et al. (2010), assigned to the new genus *Gyalectaria*. All ac-

cessions available on GenBank of representatives of the Arctomiaceae were included; they represent all species assigned to that family, except for both species of *Arctomia* described from subantarctic islands by Øvstedal and Gremmen (2001, 2006). The outgroup species (*Bacidia rosella, Lecanora intumescens* and *Toninia cinereovirens*) were chosen outside the Ostropomycetidae and within the Lecanorales (Miadlikowska et al. 2006) to avoid any putative homoplasy problem. Six new sequences were generated for this study, all belonging to the new species described in this paper (Table 1). The sequences were first aligned using MAFFT (on-line version available at http://mafft.cbrc.jp/alignment/server/) and eventually manually adjusted using MacClade v. 4.05 (Maddison and Maddison 2002). Ambiguous characters have been detected by eye and excluded from the analyses.

Three matrices were assembled: 38 species with 927 included characters for nuLSU, 38 species with 668 included characters for mtSSU and 32 species with 675 included characters for *RPB1* (part 1). Incongruence between the matrices was tested with maximum likelihood analysis using GARLI (Zwickl 2006, version 0.951 for OS X) with gaps treated as missing data, and a single most likely tree was produced. Support for the branches was estimated using bootstrap values from 1000 pseudoreplicates (all other parameters identical to the original ML search). A conflict was considered significant if a clade was supported with bootstrap support > 75% in a one-locus analysis and not in the other two. A further test for conflict was performed with LSU and *RPB1* concatenated in a single matrix versus mtSSU in another. No conflict was detected and therefore the available sequences for the three loci were concatenated. The assembled matrix is deposited in TreeBASE under the accession number 12710.

An unweighted maximum parsimony (MP) analysis was performed in PAUP* 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. A first heuristic analysis was performed using NNI (Nearest Neighbor Interchange) branch-swapping, with 1000 replicates and saving 10 trees at each step, the functions Steepest descent and MulTrees being in effect. A second analysis was performed with the 10,000 saved trees using TBR (Tree Branch Swapping), with a maximum of 200 trees saved at each step, the function Steepest descent being inactivated. A 50% consensus tree is produced, and the strength of support for individual branches was estimated using bootstrap values (MPBS) obtained from 1000 heuristic bootstrap pseudoreplicates.

A partition of six subsets was implemented in the concatenated matrix: nuLSU, mtSSU, intron in *RPB1*, and three for each *RPB1* codon position. Models of evolution for the maximum likelihood and Bayesian analysis were selected based on the Akaike Information Criterion (Posada and Buckley 2004) as implemented in Mr. Modeltest v2.3 (Nylander 2004). The selected model corresponds to the GTR model of nucleotide substitution (Rodríguez et al. 1990) including a proportion of invariable sites and a discrete gamma distribution of six rates categories. The maximum likelihood analysis was performed using RAxML-HPC2 (Stamatakis 2006) on the Cipres Gateway (Miller et al. 2010), with 1000 bootstrap pseudoreplicates. Bayesian analyses were carried out using the Metropolis-coupled Markov chain Monte Carlo method (MC^3)

Table 1. Species and specimens used for this study, with GenBank accessions numbers for the three loci examined. Newly produced sequences for *Arctomia borbonica* are in bold.

Species name	LSU	mtSSU	*1RPB*
Absconditella sp.	AY300825	AY300873	—
Acarosporina microspora	AY584643	AY584612	DQ782818
Agyrium rufum	EF581826	EF581823	EF581822
Arctomia borbonica 1 (holotype)	**JX030030**	**JX030032**	**JX030034**
Arctomia borbonica 2	**JX030031**	**JX030033**	**JX030035**
Arctomia delicatula	AY853355	AY853307	DQ870929
Arctomia interfixa	DQ007345	DQ007348	—
Arctomia teretiuscula	DQ007346	DQ007349	DQ870930
Aspicilia contorta	DQ986782	DQ986876	DQ986852
Bacidia rosella	AY300829	AY300877	AY756412
Chromatochlamys muscorum	AY607731	AY607743	FJ941910
Coccotrema pocillarium	AF274093	AF329166	DQ870940
Conotrema populorum	AY300833	AY300882	—
Diploschistes ocellatus	HQ659183	HQ659172	DQ366252
Gregorella humida	AY853378	AY853329	—
Gyalectaria diluta	GU980982	GU980974	—
Icmadophila ericetorum	DQ883694	DQ986897	DQ883723
Lecanora intumescens	AY300841	AY300892	AY756386
Neobelonia sp.	AY300830	AY300879	—
Ochrolechia parella	AF274097	AF329173	DQ870959
Ochrolechia upsaliensis	GU980986	GU980979	GU981009
Orceolina kerguelensis	AY212830	AY212853	DQ870963
Pertusaria amara	AF274101	AY300900	DQ973048
Pertusaria lactea	AF381557	AF381564	DQ870971
Pertusaria leioplaca	AY300852	AY300903	DQ870973
Pertusaria paramerae	DQ780326	DQ780293	GU981012
Pertusaria pertusa	AF279300	AF381565	DQ870978
Pertusaria pustulata	DQ780332	DQ780297	GU981013
Pertusaria subventosa	AY300854	DQ780302	DQ870981
Placopsis gelida	AY212836	AY212859	DQ870984
Protothelenella corrosa	AY607734	AY607746	DQ870988
Protothelenella sphinctrinoidella	AY607735	AY607747	DQ870989
Thamnolia vermicularis	AY853395	AY853345	DQ915599
Thelotrema subtile	DQ871013	DQ871020	DQ870998
Toninia cinereovirens	AY756365	AY567724	AY756429
Trapelia chiodectonoides	AY212847	AY212873	DQ870999
Trapeliopsis granulosa	AF274119	AF381567	DQ871001
Wawea fruticulosa	DQ007347	DQ871023	DQ871005

in MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003, Altekar et al. 2004). No priors values were assumed and gaps were treated as missing data. Four parallel runs were performed, each using four independent chains (three heated and one cold chain),

with a single tree saved every 100th generation for a total of 6,000,000 generations. The incremental heating scheme was set by default. We used TRACER v1.4.1 (Rambaut and Drummond 2007) to plot the log-likelihood values of the sample points against generation time, and determine when stationarity was achieved. Consequently the first 6,000 sampled trees were deleted as the burn-in of the chain. A majority rule consensus tree with average branch lengths was constructed for the remaining trees using the sumt option of MrBayes. Phylogenetic trees were visualized using FigTree v1.3.1 (Rambaut 2009). Branches support was considered as significant when Maximum Parsimony Bootstrap (MPBS) > 70%, Maximum Likelihood Bootstrap (MLBS) > 70% and Posterior Probabilities (PP) > 0.95.

We tested the monophyly of the genus *Arctomia* by comparing the best unconstrained tree with the best tree obtained by constraining all *Arctomia* sequences to form a monophyletic group. Trees were generated in RaxML and then tested with two methods: the Shimodaira-Hasegawa (SH) test and the Expected Likelihood Weight (ELW) test as implemented in Tree-PUZZLE 5.2. (Shimodaira and Hasegawa 2001, Strimmer and Rambaut 2002, Schmidt et al. 2002).

Results

The concatenated matrix with aligned sequences for nuLSU, mtSSU and *RPB1* has 2781 characters, out of which 511 are excluded (330 for nuLSU out of which 250 represent introns in *Bacidia rosella*, 173 for mtSSU and 8 for *RPB1*), 983 are constant, 276 are parsimony-uninformative and 1011 are parsimony potentially informative. The most parsimonious tree has the following characteristics: length = 6295 steps, CI = 0.336 and RI = 0.428. The ML analysis yielded a tree with a likelihood value of Ln = -28660.4 and length of 6.175. Parameters of the partitions were as follows: LSU — p(A)= 0.2604, p(C)= 0.2216, p(G)= 0.2980, p(T)= 0.2199 a= 0.3134, r(A-C)= 0.7438, r(A-G)= 1.8229, r(A-T)= 0.7430, r(C-G)= 0.7409, r(C-T)= 4.5270, r(G-T)= 1.0000; mtSSU — p(A)= 0.3330, p(C)= 0.1606, p(G)= 0.2136, p(T)= 0.2926, a= 0.4207, r(A-C)= 0.9284, r(A-G)= 2.9298, r(A-T)= 1.6160, r(C-G)= 0.6649, r(C-T)= 3.4571, r(G-T)= 1.0000; *RPB1* intron — p(A)= 0.2349, p(C)= 0.2056, p(G)= 0.2267, p(T)= 0,3287, a= 0.9412, r(A-C)= 6.9358, r(A-G)= 21.9085, r(A-T)= 11.1853, r(C-G)= 8.6280, r(C-T)= 19.3378, r(G-T)= 1.0000; *RPB1*, 1st codon — p(A)= 0.2778, p(C)= 0.2440, p(G)= 0.3318, p(T)= 0.1463, a= 0.4211; r(A-C)= 4.0125, r(A-G)= 5.8268, r(A-T)= 3.1946, r(C-G)= 2.7176, r(C-T)= 2907386, r(G-T)= 1.0000; *RPB1*, 2nd codon — p(A)= 0.3521, p(C)= 0.2038, p(G)= 0.2319, p(T)= 0.2122, a= 0.3474, r(A-C)= 1.7253, r(A-G)= 3.1209, r(A-T)= 0.5159, r(C-G)= 1.9509, r(C-T)= 4.4498, r(G-T)= 1.0000; *RPB1*, 3rd codon — p(A)= 0.2683, p(C)= 0.2056, p(G)= 0.2545, p(T)= 0.2716, a= 0.5667, r(A-C)= 8.7546, r(A-G)= 24.9090, r(A-T)= 4.6296, r(C-G)= 5.8128, r(C-T)= 56.3087, r(G-T)= 1.0000.

All three analyses retrieve the family Arctomiaceae as a strongly supported clade (MPBS= 81%, MLBS = 97%, PP=1) (Fig. 1). All nodes within the Arctomiaceae clade are strongly supported: *A. delicatula* and *A. teretiuscula* form a clade supported

with MLBS= 99% and PP=1.0; they further form a clade with both accessions of *A. borbonica* that is supported with MLBS = 94% and PP=1.0; *Gregorella humida* and *Wawea fruticulosa* form a clade supported with MLBS = 86% and PP= 1.0; and finally the latter is sister to the clade including all accessions of *Arctomia* (except for *A. interfixa*) in a node supported by MLBS= 95% and PP= 1.0.

SH test shows that the likelihood of the topology constraining all *Arctomia* sequences to form a monophyletic group is not significantly worse (at 0.05 significance level) than that with *Arctomia interfixa* being sister to all other accessions of the Arctomiaceae. Following that test, the monophyly of all species assigned to *Arctomia*, incl. *A. borbonica* sp. nov., cannot be rejected. The result of the ELW is the contrary: such a monophyly is rejected at 0.0473 significance level.

Discussion

The lichen family Arctomiaceae is fully recovered in our analysis (Fig. 1) and all other accessions are resolved in positions fully consistent with those published for the Ostropomycetidae (Lumbsch et al. 2005, Baloch et al. 2010, Schmitt et al. 2010), including the polyphyly of representatives of *Pertusaria* that are resolved in three distinct lineages, and the representative of the newly described genus *Gyalectaria* that is resolved as sister to the representative of *Coccotrema*. Our material is resolved without ambiguity within the Arctomiaceae. It is resolved with strong support as sister to a clade comprising the type species of *Arctomia* (*A. delicatula*). The monophyly of the three species of *Arctomia* for which DNA sequences are available, demonstrated with strong support in Lumbsch et al. (2005), is not recovered in our analysis but is not rejected by the topology tests. The assignment of our new species to the genus *Arctomia* can thus be considered legitimate. The apparent dismemberment of *Arctomia* in our analysis (with *A. interfixa* as sister to all other taxa of the Arctomiaceae) may be due to an incomplete dataset (sequences for the three loci are available for all accessions of Arctomiaceae, except for *A. interfixa* which lacks the most informative *RPB1* sequence): indeed, incomplete dataset may produce misleading results in likelihood-based analysis (Simmons 2011). However, separate analyses of LSU and mtSSU sequences yielded the same topology, with *Arctomia* paraphyletic. The status of *Arctomia interfixa* should thus be studied in more details.

Diagnostic characters for the genera recognized within the Arctomiaceae are given by Lumbsch et al. (2005). In the absence of ascomata and conidiomata, they are: thallus crustose, composed of goniocysts for *Gregorella*, fruticose for *Wawea* and crustose to coralloid or squamulose for *Arctomia*. The other two species of *Arctomia*, described by Øvstedal and Gremmen (2001, 2006) and not included in Lumbsch et al. (2005) have a thallus "placodioid" or "foliose, [...] squamulose or elongate, forming rosettes". If assigned to *Arctomia*, our new species does not match the thallus description of that genus, as its thallus is foliose and produces typical goniocysts at its margin, disintegrating into a soredioid margin (Fig. 2). We suggest the thallus of *Arctomia borbonica* is much similar to that of *Wawea fruticulosa* which has a "fruticose, olive-grey to brown" thallus (Henssen and Kantvilas 1985)

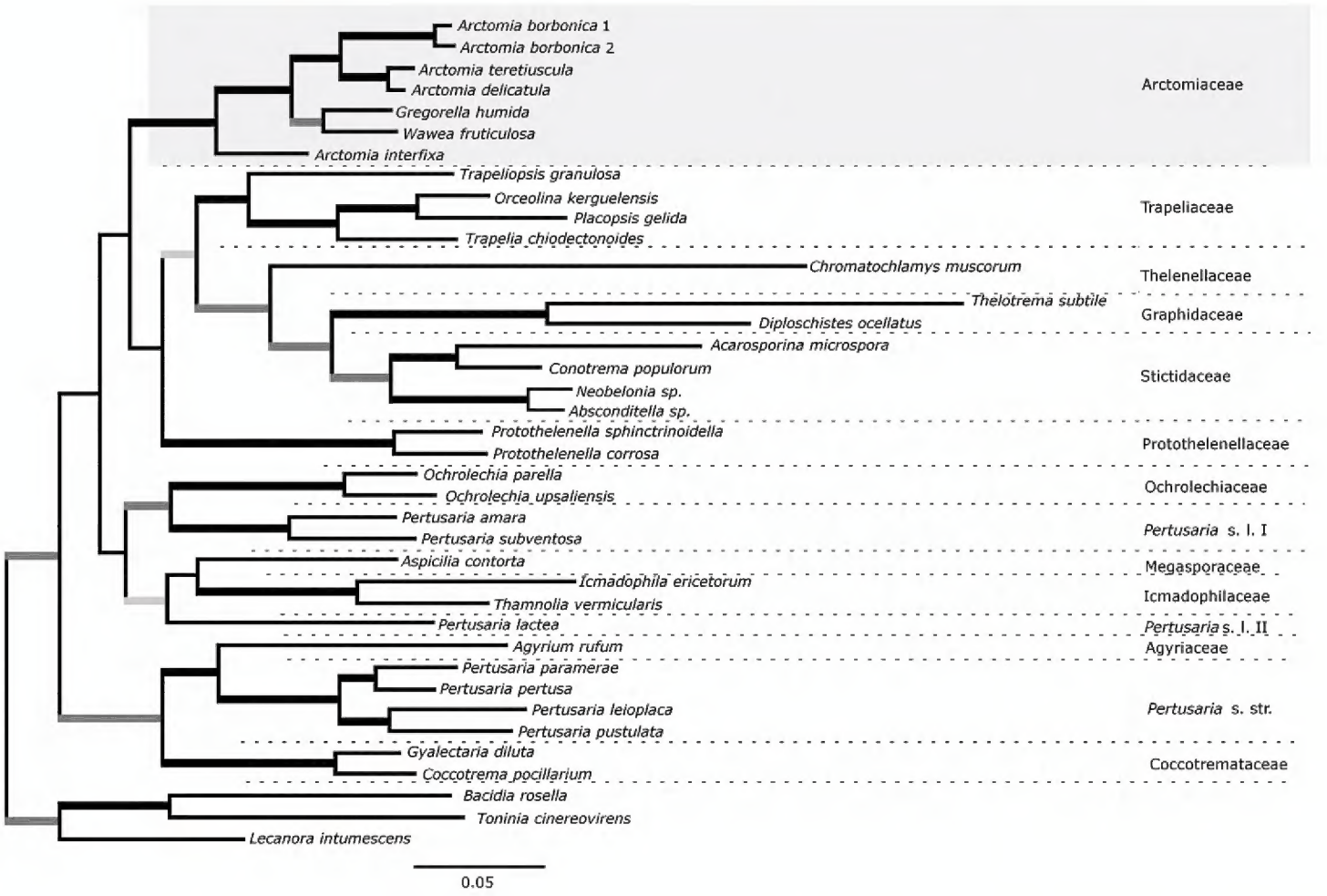

Figure 1. 50% consensus tree produced by the Bayesian analysis of a concatenated matrix with three loci (nuLSU, mtSSU and *RPB1*) with 2531 characters and highlighting the Arctomiaceae and the newly described *Arctomia borbonica*. Branches supported by MPBS and MLBS > 70% and Bayesian posterior probabilities > 0.95 are in black; those supported by MLBS >70% and Bayesian posterior probabilities > 0.95 in dark grey and those only by Bayesian posterior probabilities > 0.95 in light grey.

but with lobes flattened or at least furrowed (see fig. 2 in Henssen and Kantvilas 1985, Kantvilas and Jarman 1999). Further, the structure of the cortex is quite similar in *Wawea* (cross section and surface view: see fig. 3A–B in Henssen and Kantvilas 1985) when compared with *A. borbonica* (Fig. 2C–E). Finally, it is interesting to note that the sister species of *Wawea* is *Gregorella humida* whose thallus is entirely made of goniocysts, very similar to those produced by *Arctomia borbonica* at its thallus margin. As long as ascomata and conidiomata are not found and could provide more information, the thallus characters of *Arctomia borbonica* confuse the generic delimitations within the family.

The hypothesis of describing a new genus for *Arctomia borbonica* has been carefully assessed. Indeed, the genus as circumscribed by Henssen (1969) and Jørgensen (2007) is well-delimited and the inclusion of *A. borbonica* makes it morphologically heterogeneous. We refrained from describing a new genus because of the following points: (a) both subantarctic species recently described by Øvstedal and Gremmen (2001, 2006) in the genus, both assumed not to genuinely belonging to *Arctomia* s. str. and with generic affinities "under study", should be further studied; indeed, several characters put them aside of the genus such as a pluricellular cortex; the description of a new genus within such a small family as the Arctomiaceae is premature

in that context; (b) ascomata and conidiomata are unknown, or not yet discovered, in *A. borbonica* and thus our dataset lacks important characters (Lumbsch et al. 2005, Table 2); (c) morphological and anatomical characters may be very much misleading for phylogenetic reconstruction and sound generic delimitations as demonstrated by many studies in lichenized or unlichenized ascomycetes (Gaya et al. 2008, Lantz et al. 2011, Prieto et al. 2012, Sérusiaux et al. 2010); and (d) two statistical topology tests applied to the likelihood tree gave opposite results to assess the monophyly of *Arctomia* when including all species studied, e.g. *A. borbonica, A. delicatula, A. interfixa* and *A. teretiuscula*.

Taxonomy

Arctomia borbonica **Magain & Sérus, sp. nov.**
Mycobank: MB 800279
Fig. 2

Diagnosis. Species recognized by its foliose, usually much crumpled, blue grey to brown thallus producing goniocysts at its margins, eventually forming a soredioid margin. Ascomata and conidiomata unknown.

Type. REUNION (Mascarene archipelago). Forêt de Bébour, track starting at Gîte de Bélouve toward Piton des Neiges, 21°4'49"S, 55°31'24"E (DMS), 1850 m alt., 9 Nov 2009, wet montane ericoid tickets, N. Magain & E. Sérusiaux sn (holotype : LG).

Description. Thallus not exceeding 1 cm in diam., with distinct lobes when well-developed, lobes blue-grey to brown when dry, up to 0.2-0.3 mm wide and c. 200-400 µm thick, hardly distinguished in some specimens, with a surface typically wrinkled (even in young lobes), sometimes very much "crumpled", always developing small goniocysts, mainly at the margins but also on the upper surface; cortex (Fig. 2C–E) developed on upper and lower sides, formed by a single layer of small rounded (in cross section) and jigsaw-like (in surface view) cells, less than 5 µm thick; goniocysts (Fig. 2F) 20-80 µm across, always containing compact chains of *Nostoc* cells and covered by a layer of isodiametric to rounded cells, 2–5 µm, best developed at the lobes margins where they eventually form a typical pale brownish soredioid edge, due to cortical disintegration. Photobiont belonging to the cyanobacteria genus *Nostoc* forming chains of small rounded cells 2–5 µm in diam. Ascomata and conidiomata unknown.

Chemistry. No secondary metabolites found by TLC.

Notes. The material looks like a species in *Leptogium*, a genus belonging to the Collemataceae in the Lecanoromycetidae (Lumbsch and Huhndorf 2010). Soredia or soredioid propagules are however unknown in that genus as well as in the closely related *Collema*. *Arctomia borbonica* is easily recognized by its foliose, sometimes very much crumpled, blue grey to brown thallus, producing corticate and easily detached « goniocysts », best developed at the lobes margins, disrupting when mature and then forming a soredioid margin.

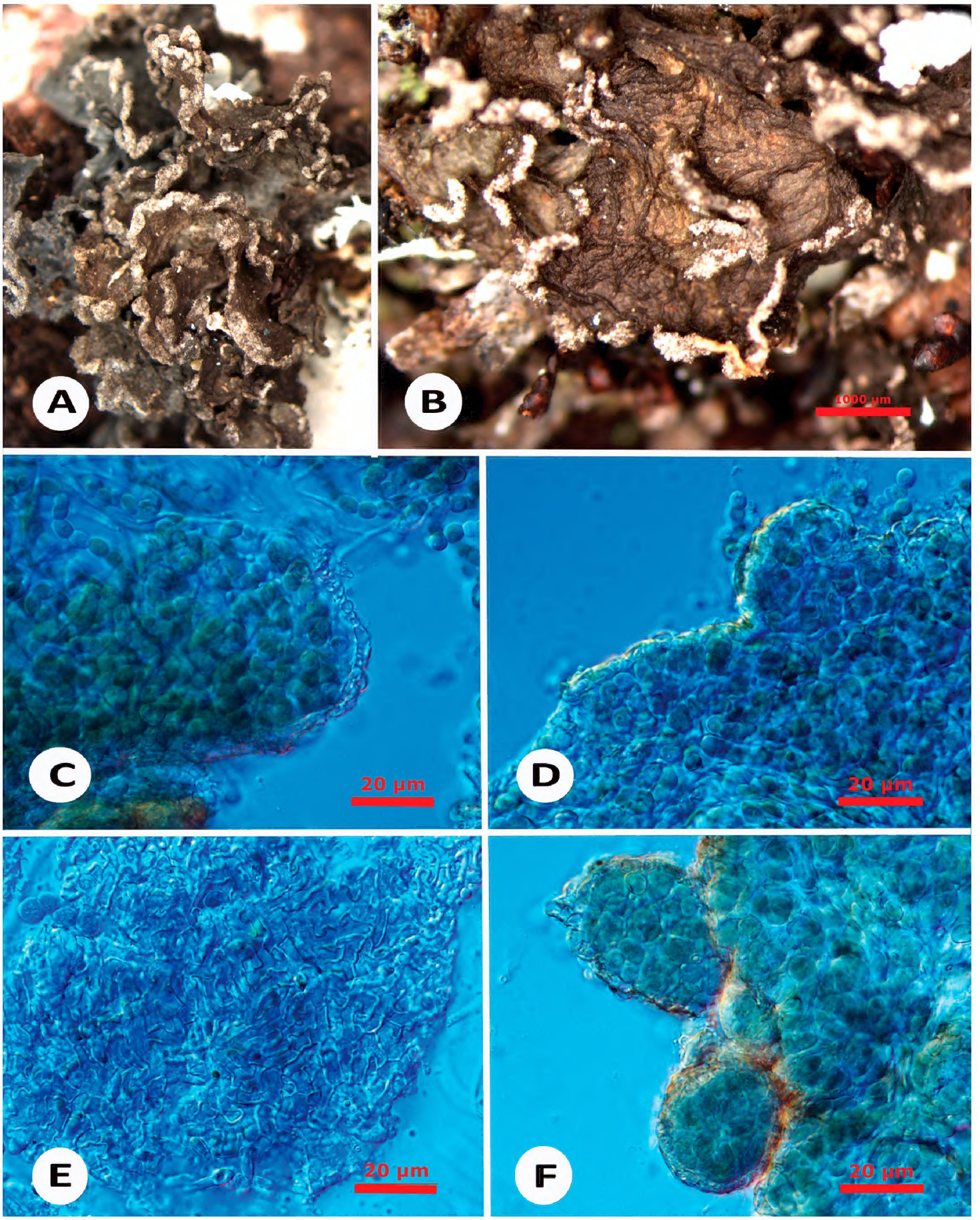

Figure 2. *Arctomia borbonica* (holotype). **A–B** macroscopic view of the thallus, with details of the wrinkled surface **B** and soredioid margin, made of disintegrating goniocysts **C–D** cross section through the thallus, showing the cortex with small, isodiametric cells, and the *Nostoc* chains **E** surface view of the cortex **F** young goniocysts formed at the lobes margins. Scale: **A–B** = 1 mm; **C–E** = 20 µm.

Distribution and ecology. *Arctomia borbonica* has been collected at three different sites on the island of Reunion in the Mascarene archipepago, incl. in highly disturbed secondary tickets with *Eucalyptus* plantations; it grows on trunks (*Eucalyptus*, *Acacia*

heterophylla) or on main stems of *Erica* tickets. It is probably widespread on the island. The two localities with natural vegetation belong to two different and typical habitats. The first one is the margin of the "Forêt de tamarins des hauts" with large boles of the endemic tree *Acacia heterophylla* (locality at the nature reserve "Roche Ecrite", at 1500 m) and corresponds to the "*Acacia* mountain forest" in Strasberg et al. (2005). The other one is the wet upper montane ericoid tickets (type locality; locality in the Bébour forest at 1800–1850 m) and corresponds to the "*Philippia* mountain ticket" in Strasberg et al. (2005). Here the vegetation does not exceed 4–5 m in height and is formed by *Erica arborescens, E. montana, Eugenia buxifolia, Agauria buxifolia, Cordyline mauritiana* (locally very abundant), *Cyathea* sp., *Phylica nitida, Astelia hemichrysa, Blechnum attenuatum*; ground is covered by very thick (up to 80 cm) layer of *Sphagnum* and other bryophytes. It is one of the most rewarding habitat for lichens on Reunion, with many interesting species, including representatives of the austral element (van den Boom et al. 2011), such as *Gomphillus morchelloides, G. pedersenii* and *Sporopodiopsis mortimeriana*.

Other specimens examined. REUNION (Masarenes archipelago). Nature reserve at Roche Ecrite, track to the summit, 20°58'6"S, 55°26'26"E (DMS), c. 1500 m alt., 4 nov 2009, montane forest dominated by *Acacia heterophylla,* N. Magain & E. Sérusiaux sn (LG). S part of the island, N of St-Philippe, near « gîte Bernard Brice », 21°20'23"S, 55°41'55"E (DMS), 650 m alt., 10 Nov 2009, *Eucalyptus* plantations and secondary tickets, N. Magain & E. Sérusiaux sn (LG).

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